Effects of L-carnitine on Action Potential of Canine Papillary Muscle Impaired by Long Chain Acyl Carnitine

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SUMMARY

It has been reported that long chain acyl carnitine accumulates in ischemic myocardium, and L-carnitine prevents ventricular arrhythmias as well as the accumulation of long chain acyl carnitine in ischemic and free fatty acid supplemented hearts. The purpose of this study was to observe the electrophysiological effects of long chain acyl carnitine, and to evaluate the protective effect of L-carnitine on the transmembrane action potential impaired by long chain acyl carnitine. Using standard microelectrode techniques, transmembrane action potentials were recorded from isolated canine papillary muscle.

Palmitoyl carnitine (0.3 mM and 0.6 mM) decreased the resting membrane potential, action potential amplitude and maximum upstroke velocity of phase 0, and shortened action potential duration and effective refractory period in a concentration-dependent manner. Application of L-carnitine (25 mM) prevented the effect of palmitoyl carnitine (0.3 mM) on the transmembrane action potential.

These results suggest that long chain acyl carnitine plays an important role in arrhythmogenesis, and that the effect is prevented by L-carnitine.

Additional Indexing Words:

Palmitoyl carnitine Free fatty acid Arrhythmogenesis

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HIGH concentration of free fatty acids (FFA) have been reported to provoke malignant dysrhythmias in ischemic heart disease[1] and in experimental animals.[2),3] Recent studies have reported that FFA cause a concentration-dependent decrease in the action potential duration and a corresponding shortening of effective refractory period.[4)-6] Several explanations have been proposed, such as a nonspecific detergent action on biomembranes,[7] uncoupling of oxidative phosphorylation,[8),9] inhibition of enzymes,[10] and interference with glycolytically derived ATP in the cytoplasm.[11),12]

Recently, much attention has been focused on the accumulation of long chain acyl CoA and long chain acyl carnitine (metabolites in FFA oxidation), as a cause of the toxic cardiac effects of myocardial ischemia and excess FFA.[13)-15] Furthermore, protective effects of exogenous L-carnitine have been demonstrated in animals with acute myocardial ischemia and supplemental excess FFA.[16)-19] The purpose of this study was to observe the electrophysiological effects of long chain acyl carnitine, and to evaluate the protective effect of L-carnitine on transmembrane action potentials impaired by long chain acyl carnitine.

METHODS

Mongrel dogs weighing 8–12 Kg were anesthetized with sodium pentobarbital (30 mg/Kg, iv). The hearts were removed and immediately placed in cooled and oxygenated Tyrode's solution. A small sample of papillary muscle was rapidly excised from the right ventricle and placed in a tissue bath. The bath was perfused with Tyrode's solution, oxygenated with 95% oxygen and 5% carbon dioxide, at a constant rate of 7 ml/min. The pH of the solution was maintained at 7.4, and the composition of the solution was as

Table I. Effects of Palmitoyl Carnitine on

	RMP (mV)	APA (mV)	dV/dT_{max} (V/sec)
Control (N=10)	−87±2	102±4	107±18
Palmitoyl Carnitine	−84±3	93±5	62±12
0.3 mM (N=5)	p<0.05	p<0.01	p<0.001
Palmitoyl Carnitine	−67±4***	78±3***	46± 7*
0.6 mM (N=5)	p<0.001	p<0.001	p<0.001

Values are mean±SD. Abbreviations: RMP=resting membrane potential; APA=action potential amplitude; dV/dT_{max}=maximum upstroke velocity of phase 0; APD_{20}=action potential duration at 20% repolarization; APD_{50}=action potential duration at 50% repolarization; APD_{90}=action potential duration at 90% repolarization; ERP=effective refractory period. P value

follows (in mM): NaCl, 137; $NaHCO_3$, 12; dextrose, 5.5; KCl, 3.0; $CaCl_2$, 2.7; NaH_2PO_4, 1.8; and $MgCl_2$, 0.5. The papillary muscle was stimulated through a bipolar Teflon-coated silver electrode placed on the papillary muscle at one end of the preparation, at twice the diastolic threshold in intensity with rectangular pulses of 2.0 msec duration. The basic cycle length was 1 sec.

Transmembrane action potentials were recorded from the papillary muscle through machine-pulled glass microelectrodes filled with 3 M KCl and with tip resistances of 10 to 15 megohms. The following parameters of the transmembrane action potential were measured: resting membrane potential (RMP), action potential amplitude (APA), and duration of action potential from the upstroke to 20, 50, and 90% repolarization (APD_{20}, APD_{50}, APD_{90}). The effective refractory period (ERP) was determined by applying a test pulse at a desired coupling interval in every eighth cycle of driving pulses. Stimulating pulses were 2 msec in duration and 4 times the diastolic threshold in intensity. The maximum upstroke velocity of phase 0 (dV/dT_{max}) was obtained by electronic differentiation and displayed on an oscilloscope. After performing the control measurements, the preparations were perfused with the Tyrode's solution containing either 0.3 mM or 0.6 mM palmitoyl carnitine and a mixture of palmitoyl carnitine (0.3 mM) and L-carnitine (25 mM). Effects of the agents were examined 30 min after starting the perfusion. Statistical analysis was performed using Student's t test and the significance was established at $p<0.05$.

Results

Effect of palmitoyl carnitine on transmembrane action potential

The effects of palmitoyl carnitine on transmembrane action potentials

Action Potentials in Canine Papillary Muscle

APD_{20}		APD_{50}		APD_{90}		ERP	
(msec)	(%)	(msec)	(%)	(msec)	(%)	(msec)	(%)
106±9		158±11		202± 9		196± 9	
88±5	81±6	135± 5	85± 4	177± 6	87±2	166±13	84±6
p<0.001		p<0.001		p<0.001		p<0.001	
66±8***	65±9*	121±13	77±10**	164±13	82±8	164±11	84±4
p<0.001		p<0.001		p<0.001		p<0.001	

represents the difference between control and palmitoyl carnitine (0.3 mM and 0.6 mM) by non-paired t-test. Asterisks represent the significance of change between conditions with 0.3 mM and 0.6 mM palmitoyl carnitine by a non-paired t-test.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

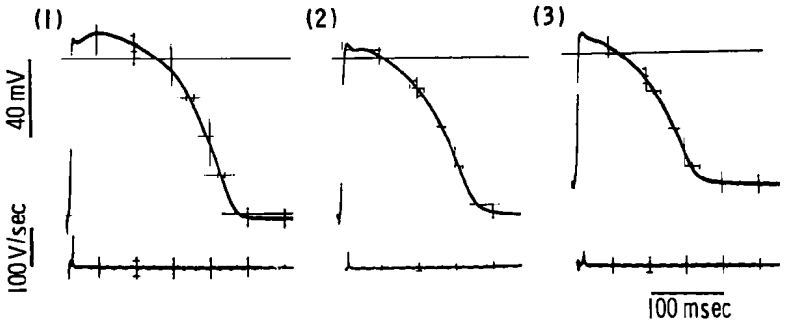

Fig. 1. Effect of palmitoyl carnitine on action potentials of canine papillary muscle. (1) control, (2) palmitoyl carnitine (0.3 mM), (3) palmitoyl carnitine (0.6 mM).

Table II. Effects of L-carnitine on Action

	RMP (mV)	APA (mV)	dV/dT_{max} (V/sec)
Control (N=10)	−86±3	103±5	100±15
Palmitoyl Carnitine 0.3 mM (N=5)	−84±3	93±5	62±12
	n.s.	p<0.01	p<0.001
Palmitoyl Carnitine 0.3 mM + L-carnitine 25 mM (N=5)	−84±5	100±8	89±23*
	n.s.	n.s.	n.s.

Values are mean±SD. P value is represented identically to Table I. Asterisks represent the significance of change between palmitoyl carnitine (0.3 mM) and palmitoyl carnitine (0.3 mM)

is summarized in Table I. A significant decrease in RMP, APA and dV/dT_{max} was observed at a concentration of 0.3 mM. Palmitoyl carnitine at a concentration of 0.6 mM caused a further decrease in RMP, APA, and dV/dT_{max}. Both the APD at all measured levels of repolarization and ERP shortened significantly at a concentration of 0.3 mM. Palmitoyl carnitine at a concentration of 0.6 mM further shortened the APD, but there was no significant change in ERP. Action potentials in a representative case are shown in Fig. 1.

Effect of L-carnitine on transmembrane action potential impaired by palmitoyl carnitine

Effect of L-carnitine on transmembrane action potentials impaired by palmitoyl carnitine was examined. Canine papillary muscle was perfused with Tyrode's solution containing palmitoyl carnitine (0.3 mM) or a mixture of palmitoyl carnitine (0.3 mM) and L-carnitine (25 mM). Table II summarizes the effects of the lower dose of palmitoyl carnitine and the mixture of

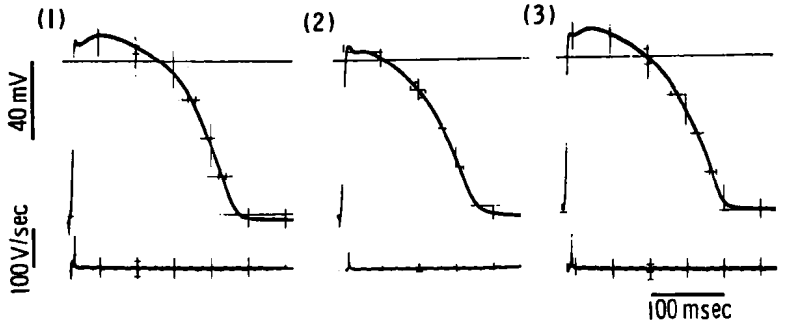

Fig. 2. Effect of L-carnitine on the action potential impaired by palmitoyl carnitine. (1) control, (2) palmitoyl carnitine (0.3 mM), (3) palmitoyl carnitine (0.3 mM) and L-carnitine (25 mM).

Potentials Impaired by Palmitoyl Carnitine

APD_{20}		APD_{50}		APD_{90}		ERP	
(msec)	(%)	(msec)	(%)	(msec)	(%)	(msec)	(%)
110±10		157±13		200±13		193±15	
88± 5	81±6	153± 5	85±4	177± 6	87±2	166±13	84±6
p<0.001		p<0.01		p<0.01		p<0.01	
96±20	87±9	149±22	96±6*	195±22	99±5***	184±26	97±4**
n.s.		n.s.		n.s.		n.s.	

and L-carnitine (25 mM) by a non-paired t-test. Abbreviations are the same as in Table I.

palmitoyl carnitine and L-carnitine on action potentials. L-carnitine prevented the decrease in dV/dT_{max}, and the shortening of APD_{50}, APD_{90}, and ERP caused by palmitoyl carnitine. As shown in Fig. 2, L-carnitine prevented the effect of palmitoyl carnitine on transmembrane action potentials.

Discussion

In this study, application of palmitoyl carnitine, an endogenous long chain acyl carnitine, causes appreciable changes in transmembrane action potentials of canine papillary muscle. RMP, APA, and dV/dT_{max} were decreased in a concentration-dependent manner, and APD and ERP were shortened. Although ionic alterations were not delineated in this study, the APD shortening evoked by palmitoyl carnitine was most prominent at 20% and 50% of full repolarization, which may result partly from the attenuation of slow inward current during the plateau.[20] The reduction in APD by

palmitate has been attributed to a depleted cytoplasmic ATP pool.[6),12)] Since palmitoyl carnitine also evokes decreases in RMP and APA, the changes do not appear to be simply due to depleted cytoplasmic ATP. It has been demonstrated that palmitoyl carnitine is a powerful inhibitor of sarcolemmal Na^+,K^+-ATPase,[21)] as well as sarcoplasmic reticulum Ca^{++}-ATPase and Ca^{++} transport.[22),23)] Changes in enzyme activity may also result from the disorganization of membrane compositions caused by the detergent action of palmitoyl carnitine.[23)] The inhibition of enzymes may cause a variable alteration of the potassium and sodium currents and the slow inward current. The resulting changes in intracellular calcium concentration may also influence the outward potassium current.[24)] Thus, an alteration in ionic currents may cause changes in transmembrane action potentials.

Since electrophysiological factors that facilitate the initiation and maintenance of reentrant dysrhythmias include alterations in myocardial conduction properties and a dispersion of recovery times,[25)] the changes in action potentials caused by palmitoyl carnitine application may induce reentrant dysrhythmias. Electrophysiological alterations induced by palmitoyl carnitine closely resemble the electrophysiological derangements characteristic of the ischemic myocardium.[26),27)] The similarity suggests that an accumulation of long chain acyl carnitine in the ischemic myocardium may be an important factor in the early electrophysiological changes that contribute to malignant dysrhythmias in acute myocardial ischemia.

Carnitine, an abundunt, normal constituent in the myocardium, functions as a carrier of activated long chain acyl groups from the cytoplasm to the intramitochondrial sites of FFA oxidation.[28)] A decrease in the myocardial free L-carnitine has been demonstrated in ischemic myocardium,[18),19)] and recent biochemical and physiological studies suggest that the administration of L-carnitine may have beneficial effects on the ischemic heart muscle.[16)-19)] We have reported that pretreatment with L-carnitine prevents the depletion in tissue levels of free carnitine and ATP, and the accumulation of long chain acyl carnitine and long chain acyl CoA in ischemic dog hearts.[18),19)] Pretreatment with L-carnitine also reduces the grade of ventricular arrhythmias induced by excess FFA.[18)] In this study, it was demonstrated that simultaneous administration of L-carnitine and palmitoyl carnitine reduces the changes in transmembrane action potentials that are impaired by palmitoyl carnitine alone. These findings suggest that L-carnitine may alleviate the arrhythmogenic action of palmitoyl carnitine, probably by decreasing the accumulation of palmitoyl carnitine in the myocardium. From these observations, it is suggested that long chain acyl carnitine may play an important role in arrhythmogenesis in the ischemic heart, and these effects may be pre-

vented by L-carnitine administration.

References

1. Oliver MF, Kurien VA, Greenwood TW: Relation between serum-free-fatty-acids and arrhythmias and death after acute myocardial infarction. Lancet **1**: 710, 1968
2. Kurien VA, Yates PA, Oliver MF: The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion. Eur J Clin Invest **1**: 225, 1971
3. Willebrands AF, Ter Welle HF, Tasseron SJA: The effect of a high molar FFA/albumin ratio in the perfusion medium on rhythm and contractility of the isolated rat heart. J Mol Cell Cardiol **5**: 259, 1973
4. Wasilewska-Dziubinska E, Czarnecka M, Beresewicz A, Lewartowski S: Influence of sodium palmitate on the cellular action potentials of the left ventricle of isolated, perfused guinea-pig heart. Acta Physiol Pol **26**: 1, 1975
5. Lüderitz B, Naumann d'Alnoncourt C, Steinbeck G: Effects of free fatty acids on electrophysiological properties of ventricular myocardium. Klin Wschr **54**: 309, 1976
6. Cowan JC, Vaughan Williams EM: The effects of palmitate on intracellular potentials recorded from Langendorff-perfused guinea-pig hearts in normoxia and hypoxia, and during perfusion at reduced rate of flow. J Mol Cell Cardiol **9**: 327, 1977
7. Kurien VA, Oliver MF: A metabolic cause for arrhythmias during acute myocardial hypoxia. Lancet **1**: 813, 1970
8. Borst P, Loos JA, Christ EJ, Slater EC: Uncoupling activity of long-chain fatty acids. Biochim Biophs Acta **62**: 509, 1962
9. Yamazaki N, Suzuki Y, Kamikawa T, Ogawa K, Mizutani K, Kakizawa N, Yamamoto M: Arrhythmogenic effects of acute free fatty acid mobilization on ischemic heart. *in* Recent Advances in Studies on Cardiac Structure and Metabolism, Vol 12, Cardiac Adaptation, ed by Kobayashi T, Ito Y, Rona G, University Park Press, Baltimore, p 271, 1978
10. Lamers JMJ, Hülsmann WC: Inhibition of $(Na^+ + K^+)$-stimulated ATPase of heart by fatty acids. J Mol Cell Cardiol **9**: 343, 1977
11. Bricknell OL, Opie LH: Effects of substrates on tissue metabolic changes in the isolated rat heart during underperfusion and on release of lactate dehydrogenase and arrhythmias during reperfusion. Circulat Res **43**: 102, 1978
12. Athias P, Pinson A, Frelin C, Padieu P, Klepping J: Comparative study on the effects of exogenous palmitate and erucate on intracellular electric properties of cultured beating heart cells. J Mol Cell Cardiol **11**: 755, 1979
13. Shug AL, Shrago E: A proposed machanism for fatty acid effects on energy metabolism of the heart. J Lab Clin Med **81**: 214, 1973
14. Shrago E, Shug AL, Sul H, Bittar N, Folts JD: Control of energy production in myocardial ischemia. Circulat Res **38** (Suppl I): 75, 1976
15. Liedtke AJ, Nellis S, Neely JR: Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. Circulat Res **43**: 652, 1978
16. Folts JD, Shug AL, Koke JR, Bittar N: Protection of the ischemic dog myocardium with carnitine. Am J Cardiol **41**: 1209, 1978
17. Liedtke AJ, Nellis SH: Effects of carnitine in ischemic and fatty acid supplemented swine hearts. J Clin Invest **64**: 440, 1979
18. Suzuki Y, Kamikawa T, Yamazaki N: Effects of L-carnitine on ventricular arrhythmias in dogs with acute myocardial ischemia and a supplement of excess free fatty acids. Jpn Circulat J **45**: 552, 1981
19. Suzuki Y, Kamikawa T, Kobayashi A, Masumura Y, Yamazaki N: Effects of L-carnitine on tissue levels of acyl carnitine, acyl coenzyme A and high energy phosphate in ischemic dog hearts. Jpn Circulat J **45**: 687, 1981

20. Trautwein W: Membrane currents in cardiac muscle fibers. Physiol Rev **53**: 793, 1973
21. Wood JM, Bush B, Pitts BJR, Schwartz A: Inhibition of bovine heart Na^+,K^+-ATPase by palmitylcarnitine and palmityl-CoA. Biochem Biophys Res Commun **74**: 677, 1977
22. Pitts BJR, Tate CA, Van Winkle WB, Wood JM, Entman ML: Palmitylcarnitine inhibition of the calcium pump in cardiac sarcoplasmic reticulum. A possible role in myocardial ischemia. Life Sci **23**: 391, 1978
23. Adams RJ, Cohen DW, Gupte S, Johnson JD, Wallick ET, Wang T, Schwartz A: In vitro effects of palmitylcarnitine on cardiac plasma membrane Na,K-ATPase, and sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} transport. J Biol Chem **254**: 12404, 1979
24. Isenberg G: Cardiac Purkinje fibres. $[Ca^{2+}]_i$ controls steady state potassium conductance. Pflügers Arch **371**: 71, 1977
25. Arnsdorf MF: Membrane factors in arrhythmogenesis. Concepts and definitions. Prog Cardiovasc Dis **19**: 413, 1977
26. McDonald TF, MacLeod DP: Metabolism and the electrical activity of anoxic ventricular muscle. J Physiol **229**: 559, 1973
27. Lazzara R, El-Sherif N, Scherlag BJ: Early and late effects of coronary artery occlusion on canine purkinje fibers. Circulat Res **35**: 391, 1974
28. Opie LH: Role of carnitine in fatty acid metabolism of normal and ischemic myocardium. Am Heart J **97**: 375, 1979